In accordance with the present amendments, claim 1 has been amended to recite that "each spot has a high coating density of one of said binding agents". This recitation, together with the recitation that "not more than 0.1 V/K moles of binding agent are present on any spot" is believed to clearly distinguish claim 1 over the combined disclosures of WO 84/01031 and Chen et al. The same is true with respect to claim 4, which also includes these same recitations.

Also, the specification has been amended to comply with arrangement and content guidelines set out in §601 of the Manual of Patent Examining Procedure.

Regarding the rejections maintained by the Examiner in the September 2, 1997 Official Action, there is submitted herewith a Substitute Declaration of the applicant, Roger P. Ekins, which includes an express claim of priority under 35 U.S.C. §119 that makes reference to PCT/GB88/00649 and UK Application No. 8803000. In view of the applicant's Substitute Declaration, those prior art rejections based on Ekins et al. (1989) and WO 88/01058 are clearly untenable, for reasons already of record, and should be withdrawn.

As for the remaining grounds of rejection, which are predicated on the combined disclosures of WO 84/01031 and Chen et al., these rejections are respectfully traversed.

The law is well settled that all claim recitations must be considered in determining non-obviousness under 35 U.S.C. §103. *In re Saether*, 181 USPQ 36 (CCPA 1974). It has long been held that when the Examiner disregards specific claim recitations that distinguish over the prior art, the rejection is improper and will be overturned. *In re Glass*, 176 USPQ 489 (CCPA 1973).

In the present case, applicant's claims 1-4 are directed to a method for determining an analyte of interest among a plurality of analytes in a liquid sample of volume V liters which utilizes a test device bearing different binding agents at a plurality of spaced apart small spots such that "each spot has a high coating density of one of said binding agents but not more than 0.1 V/K moles of binding agent are

present on any spot". The Examiner attaches no patentable significance to the quoted claim recitation, contending instead that "one of ordinary skill in the art at the time the invention was made would have sought, especially in view of the explicit teachings in WO 84/01031, to minimize the amount of binding agent so as to preclude significant alteration of the concentration of analyte in the sample. The exact value at which significant alteration is deemed to occur is a matter of choice". However, the Examiner's position in this regard totally ignores the fact that at the time the present invention was made those skilled in the art would have been deterred from using binding agent concentrations of less than 0.1 V/K, because it would be expected to lead to a drastic loss of sensitivity, resulting in assay systems requiring incubation times substantially longer than required in conventional assay techniques then in use. In support of applicant's position on this point, submitted herewith is a Declaration of Johann Berger.

Dr. Berger's declaration speaks directly to the question of what one of ordinary skill in the art would glean from the disclosure of WO 84/01031 regarding the expected effect on assay sensitivity that would be caused by reducing the concentration of binding agent to less than 0.1 V/K. As stated in paragraph 5 of Dr. Berger's declaration:

> Before the present invention was made, those of ordinary skill in the art, based on their experiences with competitive assays and the more widely practiced non-competitive assays then in use, would not have been inclined to use binding agent concentrations of less than 0.1/K (i.e., amounts of less than 0.1 V/K) for the reason that this would be expected to lead to a substantial loss of sensitivity and yield assays requiring even longer incubation times than required in conventional assay methods. Practically all recently introduced new systems, including one from our company, use microbeads as solid phase in order to increase the concentration of binder to 10 or even 100/K. I therefore believe that,

> notwithstanding the disclosure in Ekins '031, a person of ordinary skill in the art would find it surprising that the assay of the above application provides sensitivity enhancement (with shorter incubation times) by immobilizing small amounts of binding agent (less than 0.1 V/K moles) at high density in microspots. I believe it would not have been at all obvious to the person of ordinary skill in the art to do this to improve signal-to-noise ratios. As mentioned above, this runs against the conventional practice in the field of using large amounts of binding agent to obtain optimal sensitivity. Furthermore, I believe it is surprising that the sensitivity of an assay system increases as the size of the spot containing binding agent is reduced.

Thus, Dr. Berger's declaration directly refutes the Examiner's assertion that the disclosure of WO 84/01031 would have motivated those of ordinary skill in the art to practice the assay described therein with concentrations at which not more than 0.1 V/K moles of binding agent is present on any spot.

Applicant also respectfully takes exception to the Examiner's contention that the dual labelling feature of the claimed assay would have been obvious from the combined disclosures of WO 84/01031 and Chen et al.

Chen et al. cite two reasons for utilizing dual labelling in their assay. First, antibodies are labelled for the purpose of quality control to ensure that the correct amount of antibody has been attached to the assay reagent and that the resulting reagent has not been damaged in transit. The other purpose for dual labelling noted by Chen et al. is so that the signal emitted by the labelled antibody may be quantitatively detected independently of the detection of the labelled ligand bound to it. According to the disclosure of Chen et al., this renders the assay described therein self-calibrating, insofar as calibration of the fluorometer is concerned.

The first reason cited by Chen et al. for utilizing dual labelling is plainly inapplicable in the present case.

This is so because applicant's assay eliminates the requirement of careful standardization of the amount and uniformity of distribution of the binding agents affixed to the support means. A significant operational advantage of applicant's assay is that it is independent of the amount of receptor used. It logically follows, therefore, that those of ordinary skill in the art would not be motivated to use dual labelling as a way to address a perceived quality control problem when, in fact, the use of dual labelling, in accordance with the present invention, completely eliminates such a problem.

To paraphrase *In re Rinehart*, 189 USPQ 143, 149 (CCPA 1976): "Absence of any suggestion in either [WO 84/01031] or [Chen et al.] that features of the [assay] of one should be combined with features of the other to [provide an assay that is independent of the amount of receptor used] of which neither is capable requires a holding that the rejection herein [is] improper.

As for the second reason for dual labelling given by Chen et al., this reason also fails to provide the motivation necessary to modify the assay of WO 84/01031 in the manner proposed by the Examiner. Because Chen et al. is concerned with a conventional immunoassay technique and not one in which only a trace amount of antibody is used, it remains essential to the technique of Chen et al. that the amount of labelled antibody exposed to each test sample is absolutely constant when ratios are being measured, otherwise a variation in the antibody signal from sample to sample will be misinterpreted as a variation in the light-measuring efficiency of the fluorometer which Chen et al. are attempting to eliminate. This is simply not a concern in the practice of applicant's assay.

Dr. Berger's declaration also speaks to the lack of motivation for modifying the assay of WO 84/01031 to include dual labelling for the reasons advanced by Chen et al. Specifically, in paragraph 7 of the Berger declaration, it is stated:

> In my view, the reasons given by Chen et al. '126 for using dual labels have essentially no relevance to the practice of the assay of the above application. As for the first reason, i.e., ensuring affixation of the correct amount of binding agent to a test surface during manufacture, the assay of the above application is independent of the amount of binding agent used (see page 11, lines 7-23 of the above application). Thus, ensuring affixation of any specific amount of binding agent is unnecessary. In regard to the second reason, i.e., rendering the assay procedure self-calibrating with reference to instrument measurement, although this feature may be applied to any conventional assay format it provides no particular advantage for the assay of the above application. In order to properly work Chen's dual label assay requires that the function of the binder, namely, the antigen-antibody interaction, nucleic acid hybridization, etc., is strictly related to the intensity of the label on the binder. It is fairly easy to understand that this will not be the case, especially considering the stability requirements of such an assay. Therefore, this dual label approach would never be used in an assay embodying the invention of the above application.

Thus, the Berger declaration directly contradicts the Examiner's position regarding the alleged motivation for modifying the assay of WO 84/01031 to include the dual labelling feature of Chen et al.

Turning to the rejection of claims 1-8 based on WO 84/01031, Chen et al. and the commercial availability of the Bio-Rad Laser Sharp MRC-500, obviousness cannot be predicated on such a combination of references in the absence of some indication or teaching or incentive to a person of ordinary skill to combine the elements from the cited references. *In re Geiger*, 2 USPQ2d 1276 (Fed. Cir. 1987). As mentioned previously in relation to the combination of WO 84/01031 and Chen et al., applicant was the first person to realize that an assay which is independent of the amount of receptor could be carried out using a small amount of receptor in combination

with dual labelling.

It is again requested that the requirement for corrected drawings, as set forth in the October 16, 1996 Official Action, be held in abeyance pending the indication of allowable subject matter.

Entry of the foregoing amendment is respectfully requested inasmuch as it either places the application in condition for allowance, or else materially reduces the issues presented for appeal.

In view of the present amendments, the Declaration of Dr. Berger submitted herewith and the foregoing remarks, the issuance of a Notice of Allowance is in order and such action is earnestly solicited.

Respectfully submitted,

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Enclosure: Declaration of Johann Berger

U.S. Application No. 08/447,820 Examiner: M. Woodward

Filed: May 23, 1995 Group Art Unit 1813

SCHEDULE A1
SPECIFICATION AMENDMENTS

Delete the paragraph bridging pages 5-6 (i.e., page 5, line 20 through page 6, line 7, inclusive).

Page 8, immediately before the heading "Detailed description", insert the following paragraphs:

-- In arriving at the method of the invention, I have found that, generally speaking, for antibodies having an affinity constant K litres/mole for an antigen, the relationship between the antibody concentration and the fractional occupancy of the binding sites at any particular antigen concentration and the relationship between the antibody concentration and the percentage of antigen bound to the binding sites at any particular antigen concentration follow the same curves provided that the antibody concentrations and the antigen concentrations are each expressed in terms of fractions or multiples of 1/K.

Brief description of the drawing

The principle underlying the method of the invention may be better understood by reference to the accompanying drawing which is a graph representing two sets of curves plotting the relationship between antibody concentration and the fractional occupancy of the binding sites at certain prescribed antigen concentrations and the relationship between antibody concentration and the percentage of antigen bound to the binding sites at the same prescribed antigen concentrations. Each curve relates to the antibody concentration [Ab], expressed in terms of 1/K, plotted along the x-axis. For the set of curves which remain constant or decline with increasing [Ab], the y-axis represents the fractional occupancy (F) of binding sites on the antibody by

the antigen; for the second set, the y-axis represents the percentage (b%) of antigen bound to those binding sites. The individual curves in each set represent the relationships corresponding to four different antigen concentrations [An] expressed in terms of K, namely 10/K, 1.0/K, 0.1/K and 0.01/K. The curves show that as [Ab] falls F reaches an essentially constant level, the value of which is dependent on [An]. --

Page 6, line 8, delete "therefore".

U.S. Application No. 08/447,820 Examiner: M. Woodward

Filed: May 23, 1995 Group Art Unit 1813

SCHEDULE A2
CLAIM AMENDMENTS

1. (Amended) A method for determining the ambient concentration of an analyte of interest among a plurality of analytes in a liquid sample of volume V litres, comprising:

loading a plurality of different binding agents, each being labelled with a marker and being capable of reversibly binding an analyte which is or may be presented in the liquid sample and is specific for said analyte as compared to the other components of the liquid sample, onto a support means at a plurality of spaced apart small spots such that each spot has a high coating density of one of said binding agents but not more than 0.1 V/K moles of binding agent are present on any spot, where K litres/mole is the affinity constant of said binding agent for said analyte;

contacting the loaded support means with the liquid sample to be analyzed, such that each of the spots is contacted in the same step with said liquid sample, the amount of liquid used in said sample being such that only an insignificant proportion of any analyte present in said liquid sample becomes bound to said binding agent specific for said analyte;

contacting the support with a site-recognition reagent specific for each binding agent in a competitive or non-competitive technique, the site-recognition reagent being capable of recognizing either the unfilled binding sites or the filled binding sites on said binding agent, said site-recognition reagent being labelled with a marker different from the marker on said binding agent, and

measuring a ratio of signals from said markers on the site recognition reagent and the binding reagent from at least a part of the spot, from which the analyte to interest is determined.